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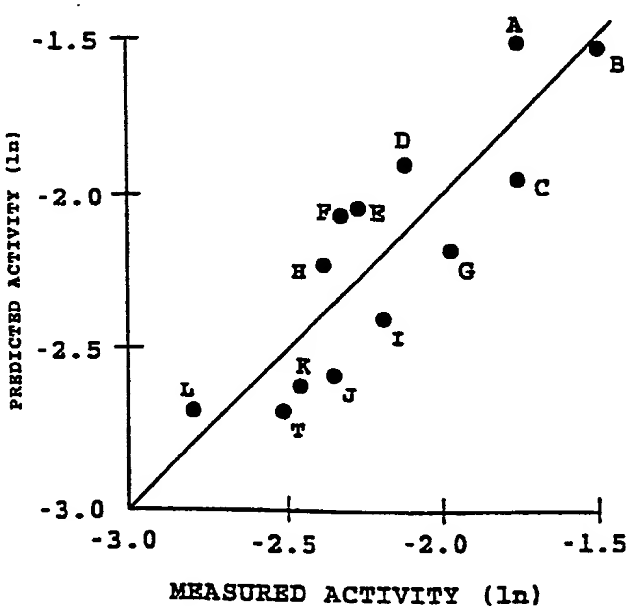

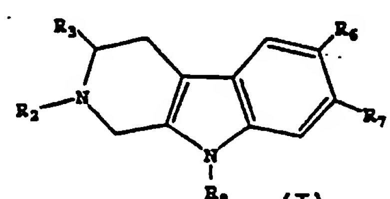

(I)

(57) Abstract

Compounds of formula (I) are useful for suppressing appetite, and for altering macronutrient preferences, wherein R_2, R_3, R_6, R_7, and R_9 are chosen such that the predicted activity, measured in terms of IC_{50}, as calculated by the quantitative structure activity relationship $\ln(1/IC_{50}) = 0.058P^2 + 0.908(\sigma + \sigma_I) - 2.647$ is greater than about -2.5, where P is the octanol/water partition coefficient as determined using the fragment method, σ is the sum of the Hammet parameters for the R_6 and R_7 substituents, σ_I is the sum of the inductive parameters for the R_3 and R_2 substituents.

* See back of page

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METHOD OF SUPPRESSING APPETITE BY ADMINISTRATION OF TETRAHYDRO-β-CARBOLINE DERIVATIVES

1. Field of the Invention

This invention relates to pharmacologic control of appetite. More specifically, this invention relates to methods for suppressing feeding behavior and modifying macronutrient preference, and compounds useful therefor.

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3. Background of the Invention

The high prevalence of obesity in the United States attests to the general failure of existing medical treatments to adequately manage the problem. The limited efficacy of existing anorectic agents when measured against possible risk factors inherent in their use currently precludes them as treatments of choice for the management of obesity. However, there is a growing awareness among both patients and the medical community that obesity is a disease that requires aggressive medical intervention. Thus, new anti-obesity agents with significantly improved performance characteristics are likely to be well received in the future.

Although the nosology of obesity and related eating disorders is not currently well defined, with its development and broadening acceptance grows the need to design safe and effective pharmacotherapies. The most commonly used weight control agents available without prescription are generally adrenergic stimulants such as phenylpropanolamine and phenethylamine derivatives. Although effective appetite inhibitors, adrenergic agents produce numerous untoward side effects, such as nervousness, irritability, insomnia, dizziness,

tachycardia, palpitations, hypertension, and the like. These side effects may be severe enough to require cessation of treatment. Kopf, DE 3,430,389, disclosed weight reduction by administering a combination of an adrenergic agent with a benzodiazepine sedative. The actual safety of such adrenergic agents is questionable, particularly in view of the 20-30% of the U.S. population suffering from hypertension. Although the non-stimulant anorectic agent fenfluramine is devoid of the psychomotor stimulant properties and abuse potential seen with stimulant-like compounds (e.g., amphetamine), it often has an inadequate clinical efficacy, and patients receiving the drug often complain of drowsiness and headache. Thus, it is apparent that none of the current anti-obesity pharmacotherapies available are particularly satisfactory.

Tetrahydro-β-carboline (THBC) has a variety of pharmacological actions and has been variously evaluated as a cholinesterase inhibitor, sedative/hypnotic, analgesic, and psychotomimetic. It competes with low affinity for brain tryptamine, imipramine, 5-hydroxytryptamine (5-HT), and spiperone binding sites, enhances depolarization- induced 5-HT efflux from brain slices, and weakly inhibits 5-HT uptake in brain synaptosomes and 5-HT oxidative deamination. It occurs naturally in mammalian brain tissue.

When THBC is administered parenterally to laboratory animals, it suppresses locomotion, exploratory activity, and conflict behavior, impairs performance on operantly conditioned learning and memory tasks, reduces seizure susceptibility, prolongs barbiturate sedation, and antagonizes specific drug-induced stereotypies. When given in high doses, THBC induces a characteristic behavioral syndrome characterized by hyperactivity, forepaw treading, body weaving, and circling. Paradoxically, THBC has been reported to reduce motor activity, induce apparent anxiety, and increase voluntary ethanol consumption when administered intraventricularly to rats. Atkinson, GB 1,183,219 disclosed its use as an analgesic.

Physiological actions of THBC include effects on endocrine secretory patterns and body temperature. Systemic administration in rodents produces a dose-dependent elevation of plasma prolactin levels, decreased serum luteinizing hormone levels, and elevated plasma corticosterone. THBC elicits significant hypothermia when administered to rats parenterally in doses of 6.25 mg/Kg or greater.

It is also known that daily oral administration of THBC produces <u>temporary</u> dose-related decrements in food and fluid intake in rats. Animals that receive average daily amounts of THBC in excess of 49 mg/Kg show significant reductions in food intake after two consecutive days of treatment; however, tolerance develops, and food consumption returns to normal by the twelfth treatment day. Smaller daily doses (less than 30 mg/Kg) do not significantly alter appetite. It is noteworthy that in Rommelspacher's report, 6 out of the 24 animals receiving 49 mg/Kg/day or greater died.

Payne et al, U.S. Pat. No. 4,336,260 disclosed the use of 1-aryl-3-carboxylic acid THBC derivatives as antidepressants.

S. Cooper disclosed that three fully-unsaturated beta-carboline derivatives exhibit hyperphagic activity, while another beta-carboline derivative exhibits anorectic activity. The hyperphagic derivatives were: ethyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate, ethyl- 5-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate, and ethyl 5-isopropoxy-4-methyl-β-carboline-3-carboxylate. The anorectic derivative was β-carboline-3carboxylic acid methyl amide (FG 7142). When injected intraperitoneally at 10.0 mg/Kg, FG 7142 reduced food consumption by partially sated rats to 30% of control.

It has been reported (Skolnick) that certain carboxy-ester beta-carboline derivatives bind with high affinity to benzodiazepine receptors. This binding may account for the ability of these compounds to antagonize the anticonvulsant, anxiolytic, and sedative properties of benzodiazepine drugs.

However, saturated derivatives such as 3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline bind with very low affinity (Skolnick; Robertson).

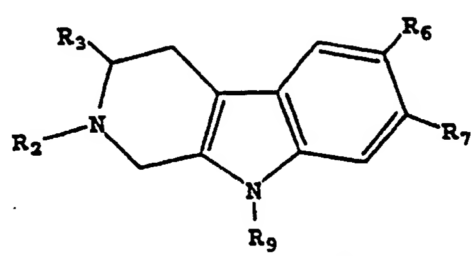
4. <u>Summary of the Invention</u>

It is therefore one objective of the present invention to provide a method of appetite suppression in mammals.

It is another objective of the present invention is to provide a composition capable of selectively suppressing carbohydrate appetite in mammals.

In accordance with the invention, it has been discovered that certain derivatives of THBC, when administered in a pharmaceutically effective dose, partially or fully suppress feeding behavior. The compounds of the invention are effective at relatively low doses. These compounds are also useful for altering macronutrient preferences (e.g., by reducing appetite for carbohydrates). The compounds of the invention exhibit very low affinity for 5-HT receptors, 5-HT uptake sites, and benzodiazepine receptors. Thus, it would appear that the compounds of the invention act by a mechanism different from that reported for saturated β-carbolines.

One aspect of the invention is the method of suppressing feeding behavior in a mammal by administering an effective amount of a compound of formula (I), where R_2, R_3, R_6, R_7 and R_9 are as described below:



(I)

The compounds used in the method exhibit strong structure-activity relationships. These relationships have been quantitatively analyzed to predict the properties of substi-

tuents which correlate with high appetite-suppression activity, e.g., activity substantially higher than that of THBC. The analysis relates the drug's biological activity, measured in terms of IC_{50} (the concentration of drug required to reduce the mammal's food intake by 50%), to the octanol/water partition coefficient of the molecule and the Hammet and Inductive electronic parameters of the R substituents.

Presently preferred compounds for use in suppression of appetite are those wherein R_2, R_3, R_6, R_7, and R_9 are selected such that the predicted activity, measured in terms of $\ln(1/IC_{50})$, is greater than about -2.5, and preferably greater than -2.1. Examples of such compounds are those in which $R_9 = n\text{-}C_5H_{11}$ and R_2, R_3, $R_6 = R_7 = H$, and $R_9 = n\text{-}C_5H_{11}$, $R_6 = CH_3$, and $R_2 = R_3 = R_7 = H$.

Another aspect of the invention is a composition useful for suppression of feeding behavior in a mammal, which comprises a pharmaceutically acceptable excipient in combination with an effective amount of a compound of formula I.

Another aspect of the invention is a method for partially suppressing appetite in a mammal, by administering a compound of formula (I) where R_2, R_3, R_6, R_7, and R_9 are as as described below. Another aspect of the invention is a composition for partially suppressing appetite in a mammal.

Brief Description of the Drawings

Figure 1 illustrates one method of synthesizing substituted 1,2,3,4-tetrahydro-ß-carbolines.

Figure 2 illustrates a second method of synthesizing substituted 1,2,3,4-tetrahydro-ß-carbolines.

Figure 3 shows a synthetic route to 1,2,3,4-tetrahydro-ß-carbolines derivatized at the aromatic nitrogen.

Figures 4A-4F show the dose-response curves for THBC compounds A-F, respectively.

Figures 5A and 5B are plots of dose response for THBC and 3-carboxy-1,2,3,4-tetrahydro-ß-carboline (5A) or 2-acetyl-1,2,3,4 tetrahydro-ß-carboline (5B).

Figure 6 shows the measured activity of derivatives A-L and THBC (T), and a plot of the structure-activity relationship calculated from the compounds; and

Figure 7 shows exemplary THBC derivatives and their predicted $\ln(1/IC_{50})$ values.

Detailed Description of the Invention

Section A establishes the definitions of several terms used herein. In Section B the general synthetic methods by which the compounds of the invention may be synthesized are described. Section C provides specific descriptions of the synthetic routes to the compounds of the invention, and presents evidence of their efficacy as appetite suppressants. Section D describes the Quantitative Structure Activity Relationship (QSAR) analysis performed with the most active appetite-suppression compounds.

A. Definitions

The term "feeding behavior" as used herein refers to food intake and associated behavior. "Partial suppression" of feeding behavior refers to reduction of feeding behavior to a level between about 20% and about 70% of control behavior.

The term "effective amount" refers to the amount of a selected compound of formula I which is necessary to cause suppression of feeding behavior. The precise amount required will vary depending upon the particular compound selected, the age and weight of the subject, route of administration, and so forth, but may easily be determined by routine experimentation. Suitable experiments are described in the Examples. In general, however, an effective amount will range from about 1 mg/Kg to about 100 mg/Kg, preferably about 2 mg/Kg to about 30 mg/Kg, more preferably about 4-12 mg/Kg. Partial suppression of feeding behavior is effected by administration of similar amounts of the appropriate derivatives of formula I.

The term "appetite-altering amount" refers to the dosage of compound required to alter the appetite for carbohydrates

experienced by the subject animal, i.e., to alter the subject's macronutrient preferences. The term "appetite-altering amount" also applies to the quantity required to effect a change in chemical dependency; in other words, a therapeutic amount in the treatment of e.g., alcohol, tobacco, narcotic or opiate addiction. The precise appetite-altering amount required will vary with the particular compounds employed, the species, age and condition of the subject to be treated. However, the amount may be determined by one of ordinary skill in the art with only routine experimentation, following methods known in the art, and disclosed below. In general, an appetite-altering amount will be roughly one half to one tenth the effective amount described above. Thus, the appetite altering amount will range from about 0.01 to about 10 mg/Kg body weight, preferably about 0.5-5 mg/Kg, and most preferably about 1 mg/Kg.

The term "pharmaceutically acceptable" refers to a compound, salt, or excipient which is not unacceptably toxic to the subject to which it is administered. Pharmaceutically acceptable salts include inorganic anions such as chloride, bromide, iodide, sulfate, sulfite, nitrate, nitrite, phosphate, and the like, and organic anions such as acetate, malonate, pyruvate, propionate, cinnamate, tosylate, and the like. Pharmaceutically acceptable excipients are described at length by E.W. Martin, in "Remington's Pharmaceutical Sciences."

B. General Synthetic Methods

Compounds of the invention may be prepared by a variety of methods known to those of ordinary skill in the art (M. Cain et al, J Med Chem (1982) 25:1081-91). Three exemplary methods are presented in Figures 1-3. Figures 1 and 2 illustrate two methods of forming the fused three-ring skeleton with substituents at the aromatic and aliphatic positions of the compounds, and Figure 3 demonstrates the addition of

a substituent R_9 at the aromatic nitrogen. Specific examples are provided in Section C below.

In Figure 1, a substituted tryptamine is cyclized by reaction with an formaldehyde under acid catalysis to form a tetrahydro-β-carboline derivative of formula I (R_9 = H). This product may be N-acylated at the aliphatic nitrogen by reaction with acetic anhydride in pyridine, with or without a co-solvent, to provide 2-acetyl derivatives of formula I wherein R_9 = H.

In Figure 2, a substituted tryptamine is cyclized by reaction with glyoxylic acid using acid catalysis to form a tricyclic carboxy product. This product may then be decarboxylated under acid catalysis to produce a tetrahydro-β-carboline derivative of formula I (wherein R_9 = H). This product may then be N-acylated at the nitrogen of the aliphatic six-membered ring (not shown) with acetic anhydride in pyridine, or without a co-solvent.

In Figure 3 the N-acylated product just described is N-alkylated at the aromatic nitrogen by reacting the compound with an alkyl halide (e.g., ethyl bromide) in an anhydrous solvent, such as dimethylformamide (DMF), and in the presence of sodium hydride (NaH). Deacylation of the compound may then be performed by standard procedures, such as refluxing under alkaline conditions.

It will be appreciated that a variety of substituted 1,2,3,4-tetrahydro-β-carbolines may be formed by the general methods just described. For example, those compounds having substituents on the aromatic ring may be formed by purchasing suitably substituted tryptamines as starting materials (as is shown in Example 1 below for compounds B, D, I, and K, L and H). Alternatively, the aromatic ring may be derivatized using techniques for the addition of substituents to aromatic compounds which are well known in organic synthesis. For example, Fridel-Crafts alkylation may be used to place aliphatic substituents on the ring.

Similarly substituents at position 3 can be made using appropriately substituted tryptamines as starting materials, or using known methods of derivatizing aliphatic rings. For example, the compound R_3 = COOH can be synthesized using tryptophan as the starting material (see compound F in Example 1 below). Compounds having substituents at the aromatic nitrogen (R_9) are easily syntesized using alkyl haides (see compounds A, B, C, and E in Example 1).

Pharmaceutical compositions containing compounds of formula I, preferably as acid addition salts, may contain one or more pharmaceutical carriers. When the carrier serves as a diluent, it may be a solid, semisolid, or liquid material acting as a vehicle, excipient, or medium for the active ingredient. Pharmaceutical unit dosage forms may be prepared for administration by any of several routes including, but not limited to, oral and parenteral (especially by intramuscular and intravenous injection, or by subcutaneous implant or transdermal administration). Representative of such forms are tablets, soft and hard gelatin capsules, powders, lozenges, chewing gums, emulsions, suspensions, syrups, solutions, sterile injectable solutions, and sterile packaged powders. Compositions containing compounds of formula I may be formulated by procedures known in the art so as to provide rapid, sustained, or delayed release of any or all of the compounds after administration.

Solid pharmaceutical excipients such as magnesium stearate, calcium carbonate, silica, starch, sugar, talc, and the like may be used with other conventional pharmaceutical adjuvants including fillers, lubricants, wetting agents, preserving agents, disintegrating agents, flavoring agents, and binders such as gelatin, gum arabic, cellulose, methylcellulose, and the like, to form admixtures which may be used as such or may be tabulated, encapsulated, or prepared in other suitable forms as noted above. The preferred liquid diluent is physiologically normal saline. A general description of formulation is given in "Remington's Pharmaceutical Sciences."

Compounds of formula I produce significant, longlasting reduction in feeding behavior when administered to mammals within 8 hours prior to meal presentation. Administration is preferably by oral dosage, but may be by transdermal application, intranasal spray, bronchial inhalation, suppository, parenteral injection (e.g., intramuscular or intravenous injection), and the like. At other doses, compounds of formula I are useful for suppressing obsessive-compulsive behavior, for altering macronutrient preferences, and for reducing craving of substances, particularly substances of abuse such as alcohol, tobacco, opiates and other narcotics.

The compound of formula I wherein $R_2 = R_6 = R_7 = R_9 = H$, and $R_3 = COOH$ is particularly useful for partial appetite suppression. This compound provides partial suppression (where food intake is 20-70% of control) over a wide dose range, in contrast to full anorectic agents which provide large suppression (> 80%) at effective dosages, and provide partial suppression over a very narrow range. As it is impractical to titrate the dosage of a full anorectic agent for each patient to achieve a particular partial appetite suppression, the method of the invention for partial appetite suppression is distinct and advantageous.

C. <u>Examples</u>

The examples presented below are provided as a further guide to the practitioner of ordinary skill in the art for preparing the THBC derivatives having the structures shown in Table 1 below.

Table 1

(Reduction of Food Intake)

Compound	Structure
T	THBC
L	R_6 = OMe
K	R_8 = Me
J	R_2 = Ac
I	R7 = F
H	R_6 = F
G	R_2 = Ac, R9 = Bz
F	R_3 = COOH
E	R_9 = Et
D	R_6 = Cl
C	R_9 = Bz
B	R_9 = n-C_5H_{11}, R_2 = Me
A	R_9 = n-C_5H_{11}

Example 1

(Preparation of Compounds)

(A) 9-(n-pentyl)-1,2,3,4-tetrahydro-ß-carboline hydrochloride was prepared as follows: Under a nitrogen atmosphere, 8.0 mmol of 2-acetyl-1,2,3,4-tetrahydroß-carboline (see J below) was dissolved in 40 mL dry dimethylformamide (DMF). The solution was stirred over ice, and NaH (2 g) was added under N2. The suspension was stirred for 1 hour, and then 1-bromopentane (9.2 mmol, 1.14 mL, 1.39 g) was added slowly to the cooled suspension. After a further 2 hours of stirring at ambient temperature, the mixture was filtered and the filtrate added to 320 mL 0.1 N HCl. Crude 2-acetyl-9-(n-pentyl)-1,2,3,4-tetrahydro-ß-carboline separated as an oil.

The oil was collected by centrifugation, washed with water, and dried over anhydrous $MgSO_4$.

A portion of the crude 2-acetyl-9-(n-pentyl)1,2,3,4-tetrahydro-ß-carboline (1.7 g) as heated at reflux for 4.5 hours in 2 N NaOH (50 mL) in methanol:water (2:3, v/v). The methanol was removed by evaporation under vacuum, and the crude 9-(n-pentyl)1,2,3,4-tetrahydro-ß-carboline free base was extracted into chloroform. This extract was dried over anhydrous $MgS0_4$, filtered, and the filtrate evaporated under vacuum. The hydrochloride salt was prepared by passing dry HCl through a solution of the crude free base in diethyl ether. The resulting precipitate was filtered and recrystallized from acetonitrile to yield pure 9-(n- pentyl)-1,2,3,4-tetrahydro-ß-carboline.

(B) 6-Methyl-9-(n-pentyl)-1,2,3,4-tetrahydro-ß-carboline hydrochloride was prepared as follows: 5-Methyltryptamine hydrochloride (Sigma) was condensed with glyoxylic acid as described in part B above, and decarboxylated according to the procedure described in part C. The product, 6-methyl-1,2,3,4-tetrahydro-ß- carboline hydrochloride was recrystallized from ethanol:water.

Under a nitrogen atmosphere, 7.7 mmol of the above-described 6-methyl compound (1.71 g) was disolved in 40 mL dry dimethylformamide (DMF). The solution was stirred over ice, and NaH (2 g) was added under N_2. The suspension was stirred for 1 hour, and then 1-bromopentane (9.2 mmol, 1.14 mL, 1.39 g) was added slowly to the cooled suspension. After a further 2 hours of stirring at ambient temperature, the mixture was filtered and the filtrate added to 320 mL 0.1 N HCl. Crude 6-methyl-9-(n-pentyl)-1,2,3,4-tetrahydro-ß-carbolineseparated as an oil. The oil was collected by centrifugation, washed with water, and dried over anhydrous $MgSO_4$.

The hydrochloride salt was prepared by passing dry HCl through a solution of the crude free base in diethyl ether. The resulting precipitate was filtered and recrystallized from

acetonitrile to yield pure 6-methyl-9-(n-pentyl)-1,2,3,4-tetrahydro-ß-carboline.

(C) 9-benzyl-1,2,3,4-tetrahydro-ß-carboline hydrochloride was prepared as follows: Under a nitrogen atmosphere, 8.0 mmol of 2-acetyl-1,2,3,4-tetrahydro-ß-carboline (see J below) was disolved in 40 mL dry dimethylformamide (DMF). The solution was stirred over ice, and NaH (2 g) was added under N_2. The suspension was stirred for 1 hour, and then benzyl-bromide (9.2 mmol, 1.56 g) was added slowly to the cooled suspension. After a further 2 hours of stirring at ambient temperature, the mixture was filtered and the filtrate added to 320 mL 0.1 N HCl. Crude 2-acetyl-9-benzyl-1,2,3,4-tetra-hydro-ß-carboline separated as an oil. The oil was collected by centrifugation, washed with water, and dried over anhydrous $MgSO_4$.

A portion of the crude 2-acetyl-9-benzyl-1,2,3,4-tetra-hydro-ß-carboline (1.7 g) was heated at reflux for 4.5 hours in 2 N NaOH (50 mL) in methanol:water (2:3, v/v). The methanol was removed by evaporation under vacuum, and the crude 9-benzyl1,2,3,4-tetrahydro-ß-carboline free base was extracted into chloroform. This extract was dried over anhydrous $MgSO_4$, filtered, and the filtrate evaporated under vacuum. The hydrochloride salt was prepared by passing dry HCl through a solution of the crude free base in diethyl ether. The resulting precipitate was filtered and recrystallized from acetonitrile to yield pure 9-benzyl--1,2,3,4-tetrahydro-ß-carboline.

(D) 6-chloro-1,2,3,4-tetrahydro-b-carboline hydrochloride was prepared as follows: 6-chlorotryptamine hydrochloride (Sigma) was condensed with glyoxylic acid as described in part B, and decarboxylated according to part C. The product, 6-chloro-1,2,3,4-tetrahydro-ß-carboline hydrochloride was recrystallized from ethanol:water.

(E) 9-ethyl-1,2,3,4-tetrahydro-β-carboline hydrochloride was prepared as follows: Under a nitrogen atmosphere, 8.0 mmol of 2-acetyl-1,2,3,4-tetrahydro-β-carboline (see J below) was disolved in 40 mL dry dimethylformamide (DMF). The solution was stirred over ice, and NaH (2 g) was added under N_2. The suspension was stirred for 1 hour, and then ethylbromide (9.2 mmol, 1.56 g) was added slowly to the cooled suspension. After a further 2 hours of stirring at ambient temperature, the mixture was filtered and the filtrate added to 320 mL 0.1 N HCl. Crude 2-acetyl-9-ethyl-1,2,3,4-tetrahydro-β-carboline separated as an oil. The oil was collected by centrifugation, washed with water, and dried over anhydrous $MgSO_4$.

A portion of the crude 2-acetyl-9-ethyl-1,2,3,4-tetrahydro-β-carboline (1.7 g) was heated at reflux for 4.5 hours in 2 N NaOH (50 mL) in methanol:water (2:3, v/v). The methanol was removed by evaporation under vacuum, and the crude 9-ethyl1,2,3,4-tetrahydro-β-carboline free base was extracted into chloroform. This extract was dried over anhydrous $MgSO_4$, filtered, and the filtrate evaporated under vacuum. The hydrochloride salt was prepared by passing dry HCl through a solution of the crude free base in diethyl ether. The resulting precipitate was filtered and recrystallized from acetonitrile to yield pure 9-ethyl-1,2,3,4-tetrahydro-β-carboline.

(F) (±) 3-carboxyl-1,2,3,4-tetrahydro-β-carboline was prepared as follows: (±) tryptophan benzyl ester hydrochloride (3.308 g, 10 mmol, obtained from Bachem) was suspended in 0.05N H_2SO_4 (18.8 mL) with stirring, and 37% formaldehyde (0.938 mL) was added. The reaction mixture cleared, followed by crystallization of the product. Stirring continued for 24 hours before the product was filtered off and dried over P_2O_5. The crude material was recrystallized from ethanol/water and the crystals were dried over P_2O_5.

10 mmol of the product was next dissolved in 30 mL 2.5 N HCl and refluxed for one hour. Upon cooling the product precipitated from the reaction mixture, was isolated by filtration and dried under vacuum. The crystals were then dissolved in water, the pH was raised to 12 using 10 N NaOH, and the precipitated free base was centrifuged, washed with water, and dried under vacuum.

(G) 2-acetyl-9-benzyl-1,2,3,4-tetrahydro-β-carboline was prepared as follows: Under a nitrogen atmosphere, 8.0 mmol of 2-acetyl-1,2,3,4-tetrahydro-β-carboline (1.71 g) was dissolved in 40 mL dry dimethylformamide (DMF). The solution was stirred over ice, and NaH (2 g) was added under N2. The suspension was stirred for 1 hour, and then benzylbromide (9.2 mmol, 1.56 g) was added slowly to the cooled suspension. After a further 2 hours of stirring at ambient temperature, the mixture was filtered and the filtrate added to 320 mL 0.1 N HCl. Crude 2-acetyl- 9-benzyl-1,2,3,4-tetrahydro-β-carboline separated as an oil. The oil was collected by centrifugation, washed with water, and dried over anhydrous $MgSO_4$. The product was purified by standard chromatographic procedures using a 60:40 mixure of ether:hexane.

(H) 6-fluoro-1,2,3,4-tetrahydro-b-carboline hydrochloride was prepared as follows: 6-fluorotryptamine hydrochloride (Sigma) was condensed with glyoxylic acid as described in part B, and decarboxylated according to part C. The product, 6-fluoro-1,2,3,4-tetrahydro-β-carboline hydrochloride was recrystallized from ethanol:water.

(I) 7-fluoro-1,2,3,4-tetrahydro-b-carboline hydrochloride was prepared as follows: 5-fluorotryptamine hydrochloride (Sigma) was condensed with glyoxylic acid as described in part B, and decarboxylated according to part C. The product, 5-fluoro-1,2,3,4-tetrahydro-β-carboline hydrochloride was recrystallized from ethanol:water.

(J) 2-acetyl-1,2,3,4-tetrahydro-β-carboline was prepared as follows: 1 g of 1,2,3,4-tetrahydro-β-carboline (Sigma) was dissolved in a minimum of ethyl acetate, and pyridine (3 mL) was added along with acetic anhydride (1.5 mL). After 30 minutes, the mixture was dried and the resulting 2-acetyl-1,2,3,4-tetrahydro-β-carboline was recrystallized from acetone.

(K) 8-methyl-1,2,3,4-tetrahydro-β-carboline was prepared as follows: 8-Methyltryptamine hydrochloride (Sigma) was condensed with glyoxylic acid as described in part B above, and decarboxylated according to the procedure described in part C. The product, 8-methyl-1,2,3,4-tetrahydro-β-carboline hydrochloride was recrystallized from ethanol:water.

(L) 6-methoxy-1,2,3,4-tetrahydro-β-carboline was prepared as follows: 5-hydroxytryptamine hydrochloride (Sigma) was condensed with glyoxylic acid as described in part B above, and decarboxylated according to the procedure described in part C. The product, 6-hydroxy-1,2,3,4-tetrahydro-β-carboline hydrochloride was dried over $MgSO_4$, dissolved in anhydrous and reacted with an equivalent of methyl iodide. The final product, 6-methoxy-1,2,3,4-tetrahydro-β-carboline, was recrystallized from ethanol:water.

Example 2
(Reduction of Food Intake)

Dose ranging studies showed that compounds of formula I significantly reduced food intake when administered parenterally to rats in amounts appreciably lower than 25 mg/Kg.

Adult male rats weighing between 250 and 300 g were acclimated to laboratory conditions for a period of 4-5 days, during which they were allowed unrestricted access to food (Ralston-Purina #5001M) and water. All animals were housed

in individual cages. The animal facility was maintained on a 12:12 hr light-dark schedule at 22°C.

Fasted animals were sorted into groups of 10-12 each by weight and baseline food intake. Each was then given saline containing 0-32 mg/Kg of a compound of the invention (or THBC) by intraperitoneal injection. After 20 minutes, animals were allowed access to food and water. Cumulative food intake was measured at 1 hour post-injection.

The measured food intake was plotted as a function of the natural logarithm of the drug concentration (ln(Dose)) given to the animal for each of the compounds A-L above. A dose response curve of the functional form: Intake = $\alpha + (1 - \alpha)/[1 + \exp(\beta \cdot \gamma \ln(\text{Dose}))]$ was fit to each of the data sets using the NONLIN feature of the Systat™ statistical analysis software package for the Macintosh™ (Systat, Evanston, Il). The data and the fitted curves for compound A-F of the invention (as defined in Section B) are shown in Figures 4A-4F, respectively.

The dose level which caused a 50% reduction in food intake, hereinafter referred to as the IC_{50} of the compound, was then determined by the formula $IC_{50} = [(\text{Intake}(t_0) - \alpha)/(1 - \text{Intake}(t_0))]^{1/\gamma\alpha} + \beta/\gamma$, where Intake($t_0$) is the measured intake of food at time = 0, and the coefficients α, β, and γ were obtained from the curve fitting procedure described above. The IC_{50} values for THBC and thirteen other compounds, including those of the invention, were calculated and are shown in Table 2.

In the examples herein, compounds were administered parenterally. In clinical usage as an anorectic agent in mammals, particularly humans, the oral, intranasal, or transdermal routes of administration would be preferred. In the case of intraperitoneal administration in rodents, amounts as low as about 1.5 mg/Kg of body weight have been shown to achieve effective significant appetite suppression.

Table 2

(Reduction of Food Intake)

Compound	Structure	IC_{50} (mg/Kg)
T	THBC	14.58
L	$R_6 = OMe$	14.80
K	$R_8 = Me$	13.61
J	$R_2 = Ac$	13.25
I	$R_7 = F$	10.65
H	$R_6 = F$	8.76
G	$R_2 = Ac$, R9 = Bz	8.30
F	$R_3 = COOH$	7.68
E	$R_9 = Et$	7.61
D	$R_6 = Cl$	7.05
C	$R_9 = Bz$	6.74
B	$R_9 = n\text{-}C_5H_{11}$, $R_2 = Me$	5.01
A	$R_9 = n\text{-}C_5H_{11}$	5.00

<u>Example 3</u>

(Partial Appetite Suppression)

Compound F ($R_2 = R_6 = R_7 = R_9 = H$, $R_3 = COOH$) demonstrated qualitatively different dose-response characteristics from THBC. Unlike THBC, compound F will not depress feeding behavior over a comparatively large feeding range. This feature, termed "partial suppression" of feeding behavior, greatly increases the margin of safety, as overuse of the compound will not induce fatal anorexia.

Reduction in food intake over one hour was measured against the dose per unit body weight for THBC and compound F. The results are shown in Figure 5A, which compares compound F with THBC.

The data demonstrate that THBC effected little feeding depression at low dosage, but relatively complete feeding depression at higher dosage, with a comparatively narrow range of concentration in the transition region. In contrast, compound F exhibited moderate feeding depression over a broad dosage range, beginning at dosages lower than those required for THBC, and extending past dosages at which THBC caused complete feeding cessation. The same behavior was observed for compound J (R_2 = Ac, $R_3 = R_6 = R_7 = R_9$ = H).

Example 4

(Alteration of Macronutrient Preference)

This example demonstrates alteration of macronutrient preference in rats.

Sixty adult male rats (Sprague-Dawley, 225-300 g) were acclimated to laboratory conditions for a period of 10 days, during which they were allowed unrestricted access to food (Ralston-Purina #5001M) and water. All subjects were housed in individual cages, and the animal facility was maintained on a 12:12 hour light:dark cycle at 24-27°C.

The animals were assigned to six groups (ten per group), then allowed to consume, ad libitum, one of two isonitrogenous test diets containing either 75% or 25% carbohydrate. After three days, the food jars were removed. After an additional twenty-four hours, the rats were administered either a saline solution, or a compound of formula I (at a dose of 1.5 or 3.0 mg/Kg of body weight); then the rats were given immediate access to the test diets. The cumulative amount, in grams (mean ± SEM), of each diet consumed by the experimental and control groups during the subsequent two-hour period was recorded.

The results indicate that the animals receiving a compound of the invention consumed significant less of the high carbohydrate diet than the controls, but both groups consumed equivalent amounts of the low carbohydrate diet.

Thus, compounds of the invention selectively suppress carbohydrate cravings when administered at doses lower than the dosage effective for global reduction in appetite. This demonstrates the utility of the present invention as a method for reducing substance cravings per se, insofar as food cravings model clinical syndromes in which there is excessive preoccupation with, or urges for, specific habituating substances (Glassman et al., Science (1984) 226:864). Accordingly, this Example may be taken as evidencing efficacy in the treatment of alcohol, tobacco, or drug (particularly opiate) addiction.

Example 5

(Formulations)

(A) A representative capsule formulation is prepared as follows:

Compound	50.0 mg
starch	3.0 mg
magnesium stearate	3.0 mg
lactose	110.0 mg
polyvinylpyrrolidone	3.0 mg

The compound of formula I, starch, magnesium stearate, lactose, and polyvinylpyrrolidone are granulated in methanol, dried, and loaded into capsules. Alternatively, the mixture may be tableted by standard methods.

(B) An oral suspension is prepared as follows:

Compound	50.0 mg
fumaric acid	5.0 g
NaCl	2.0 g
methyl paraben	0.1 g
granulated sugar	25.5 g
sorbitol (70% aq)	12.9 g
Veegum K	1.0 g
flavorings	0.035 mL
colorings	0.5 mg
distilled water	100.0 mL

The components are mixed together and stored in a sealed vessel.

(C) A formulation suitable for parenteral administration is prepared as follows:

Compound		40.0 mg
KH2P04 buffer (0.4M)		2.0 mL
KOH (1N)	qs	pH 7.0
water	qs	20.0 mL

The components are mixed together and stored under sterile conditions.

D. <u>Quantitative Structure Activity Relationships</u>

The compounds of the invention exhibit a number of structural and electronic similarities. Using the methods of quantitative structure activity relationship (QSAR) analysis (Hansch), these features have been expressed in a mathematical formula which relates the IC_{50} of the compound to its solubility properties and the electronic properties of the substituents.

It is well known that the reactivity, both *in vivo* and *in vitro*, of many chemicals depends on a relatively small number of physical parameters. These parameters include: the ability of the compound to move between hydrophilic and hydrophobic environments (P), and the effects, both electronic and steric (σ, E_s), of substituents on the compound. Using these parameters, the biological activity of a large number of diverse chemical compounds has been predicted using a mathematical expression of the form:

$$\ln(1/\text{activity}) = a\ln(P)^2 + b\ln(P) + c\sigma + dE_s \quad (1).$$

By determining the parameter values for each of the members of a group of active compounds, the coefficients of

Equation (1) can be found by multivariate linear least squares fit of (1) to the measured activity. From the resulting equation, the activity of other compounds can be determined. Each of the components of Equation (1) will now be discussed.

Activity is the chemical concentration required to achieve a defined effect. For example, activity may represent the concentration of a drug at which 50% of the recipients die (LD_{50}) or the concentration required to reduce some biological activity by 50% (IC_{50}).

P is a measure of the partitioning of the chemical species between lipophilic and hydrophilic environments, typically the chemical's partitioning between octanol and water (Hansch). P is defined as (concentration of species in lipophilic phase)/(concentration of species in hydrophilic phase). This ratio may be measured directly using radioisotope labelling experiments or chromatography, or it may be calculated.

Radioisotope methods are performed by adding the radioisotopically labelled compound to a vessel containing a biphasic mixture of the hydrophobic and hydrophilic solvents, e.g., octanol and water, then agitating the solution for a fixed period. The radioisotope label should not alter the physico-chemical properties of the compound; thus isotopes of atoms alredy present on the compound should be used (e.g., tritium for hydrogen). After mixing, the phases are isolated, and the amount of radioactivity in each phase is measured. Since the concentration of compound in the solution is proportional to the quantity of radioactivity measured, P is easily determined.

Another, more convenient, method of direct measurement involves measuring the chromatographic retention factor R_f of the compound.

P may also be calculated conventionally from tabulated factors by the fragment method (Hansch, Leo). Based on the large number of compounds whose ln(P) values are known, fragment values of ln(P) for a variety of atoms, groups and bonds

have been tabulated (Hansch, Leo). In the fragment method, the value of ln(P) for a compound can be determined by decomposing its molecular structure into a set of molecular fragments, referencing the tabulated fragment values for each of the fragments and the values for the bonding arrangement of the molecule, and summing those values. As defined herein, the "fragment method" refers to the partition coefficient calculation based on fragment values which have been reported (Hansch).

The parameter σ is a measure of the effect that substituents have on the electronic character of the molecule, usually in terms of change in the reactivity at a second substituent. The most commonly used is the Hammet σ parameter, which is well known in the chemical arts and has been successfully employed to predict chemical behavior (March). Similar indices such as the Swain and Lupton σ_I and σ_R parameters have also been used in QSAR analysis. Many compilations of the various σ-type electronic parameters can be found in the chemical literature (Jaffe). Techniques to calculate σ which employ Huckel molecular orbital theory also exist (Dewar).

All of the tabulated Hammet σ values are determined by measuring the effect of substituents on the dissociation of benzoic acid in water at 25°C (March). These benzene-based parameters are applicable to other aromatic ring systems -- even those having heteroatoms (Jaffe). Additionally, the aromatic ring to which the substituent is attached need not contain a carboxyl group. For example, the reactivity of substituted anilines towards amide formation has been successfully correlated with the substituent's σ value (Jaffe).

The σ parameter contains the effects of the substituent on the pi-electron system of the molecule as well as the through-space inductive effect on the reference substituent (March). These effects have been separated into resonance (pi-electron system effects) and inductive (through-space effects) components, denoted σ_R and σ_I, by Swain and Lupton

(March). Thus the σ_R and σ_I can be considered as a reformulation of the Hammet σ. It will be appreciated that as the inductive effect of a substituent does not involve resonance, the σ_I parameter is relevant to substituents of saturated (non-aromatic) molecules as well.

Since the effect of a substituent varies according to whether it is meta or para to the carboxyl group of benzoic acid, there is a different value of σ for each location. The Hammet σ values for a given substituent are referred to herein as the para (meta) position Hammet parameter for the R_n substituent, and will be written as σ_n. However, the σ_R or σ_I do not have such distinctions; hence the R_n^{th} substituent will be expressed hereinafter as the Resonance (Inductive) parameter for the R_n substituent, and will be written as $\sigma_{nR(I)}$.

E_s is the Taft steric parameter, which measures the bulkiness of substituents. The parameter is defined in terms of the effect of a substituent on the rate of hydrolysis of ortho-substituted benzoyl esters (March). As with the Hammet parameter, other measures also exist, but these have been shown to strongly correlate with each other (Gallo). Steric parameters may also be calculated, using the STERIMOL program (Verloop) or the calculated Van der Waals volume of the substituent (Hansch), using, for example, the program GEPOL/87 which is available from the Quantum Chemistry Program Exchange, Department of Chemistry, University of Indiana (program #554).

The actual QSAR analysis comprises calculating, measuring, or referencing the values of the above-described parameters for a family of compounds having a standard molecular skeleton and varying substituents. The parameters are tabulated and a multivariate linear regression (Tabachnick) is performed to identify the values of the coefficients. These techniques are well known in experimental science, and many commercially available software packages exist which are capable of performing such regressions, e.g., Systat™, SAS™,

SPSS™. These packages are available for both mainframe and personal computers.

Typically the amount of measured data required to perform an accurate regression is at least five times the number of parameters which are to be fitted (Tabachnick). To obtain the most robust result, the regression is repeated, with number parameters included in the regression being varied until the most statistically significant result which depends on the fewest number of parameters is obtained.

The significance of the regression is measured in terms of the multiple correlation coefficient R and the square of multiple correlation coefficient R^2. The adjusted squared multiple correlation coefficient, called Adjusted R^2, measures the accuracy of the regression for data not included in the regression, and the value p which measures the probability that the results of the regression are likely to be due to random events. The correlation coefficients indicate how much of the observed data is accounted for by the model; thus the ideal values of these indicator is ±1 (-1 indicates an inverse relationship). p determines whether or not the regression is statistically meaningful. Values of p which are less than 0.05 are considered to indicate statistically significant regressions (Tabachnick). With respect to the individual coefficients of the regression, their significance is also measured with a p value which is interpreted an a manner identical to that just described for the regression.

Such a QSAR analysis as just described was performed on 12 compounds which demonstrated activity greater than THBC, as measured in terms of IC_{50} as described. The ln(P) values for each of the compounds was calculated using the fragment method described above. The electronic parameters for the substituents were handled in the following manner: If the substituent was attached to the aromatic ring, then the Hammet σ value given in March for the substituent corresponding to the relative location of the substituent to the aromatic nitrogen was used. If the substituent was attached to the

saturated (non-aromatic) ring the Swain-Lupton σ_I value of the substituent given in March and Leo was applied to model inductive effects. Taft E_s values given in Unger were used for all substituents. If a molecule had multiple substituents, then for each parameter the values for the substituents were summed.

The regression analyses were performed using the MGLH (Multiple General Linear Hypothesis) routine of the Systat™ statistical analysis package on a Macintosh IIcx computer. Various combinations of parameters were fit to the data, and each resulting regression was analyzed using the criteria described above. The best equation, i.e., the equation employing the fewest number of parameters and having the highest value of R, is given below:

QSAR Results

$$\ln(1/IC_{50}) = 0.058(\pm 0.009)\ln(P)^2 + 0.908(\pm 0.293)(\sigma + \sigma_I) - 2.647(\pm 0.093)$$

$R = 0.894$, $R^2 = 0.800$, Adjusted $R^2 = 0.760$, $p = 0.000$*

*The p values for the regression coefficients were 0.000, 0.010, and 0.000 respectively.

The R^2 value indicates that the regression accounts for 80% of the variation in the data, i.e., the predicted activity, measured in terms of $\ln(1/IC_{50})$, is within 20% of the predicted activity. This is illustrated in Table II below which lists the measured and predicted IC_{50} values for the compounds used in the regression. A plot of the measured $\ln(1/IC_{50})$ against the predicted $\ln(1/IC_{50})$ is shown in Figure 6. It will be appreciated that the points fall within 10% of a line having unit slope, which is indicates that the model equation accurately describes the variation in the data. In terms of the predictive ability of the model, the Adjusted R^2

indicates that 76% of the variance in the data for compounds not used in the regression is accounted for by the model.

Table 3
(Reduction of Food Intake)

Compound	Structure	IC_{50} (mg/Kg) (Measured)	IC_{50} (mg/Kg) (Predicted)	$(1/IC_{50})$ Measured	$(1/IC_{50})$ Predicted
T	THBC	14.58	12.18	-2.68	-2.5
L	R_6 = OMe	14.80	16.21	-2.70	-2.89
K	R_8 = Me	13.61	11.88	-2.61	-2.48
J	R_2 = Ac	13.25	10.94	-2.58	-2.39
I	R_7 = F	10.65	8.68	-2.37	-2.16
H	R_6 = F	8.76	10.30	-2.17	-2.33
G	R_2 = Ac, R_9 = Bz	8.30	7.42	-2.12	-2.00
F	$1R_3$ = COOH	7.68	9.56	-2.04	-2.26
E	R_9 = Et	7.61	9.24	-2.03	-2.22
D	R_6 = Cl	7.05	8.31	-1.95	-2.12
C	R_9 = Bz	6.74	5.74	-1.91	-1.75
B	R_9 = n-C_5H_{11}, R_2 = Me	5.01	4.82	-1.61	-1.57
A	R_9 = n-C_5H_{11}	5.00	5.89	-1.61	-1.77

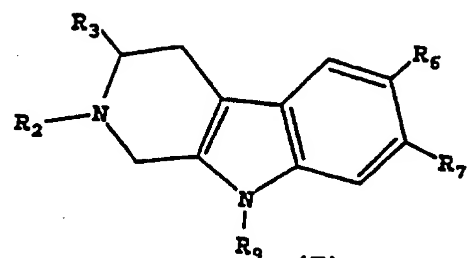
Examples of the model's ability to discern active molecules are presented in Figure 7. Compounds M and N bear close structural relationships to the compounds of the invention, but are predicted to have values of $\ln(1/IC_{50})$ less than -2.5; this is corroborated by the measured IC_{50} values. In contrast, compounds O-Q are expected to be active based on the similarity of their structures to compound D ($R_2 = R_3 = R_7 = R_9$ = H, R_6 = Cl), and all of the predicted activities are substantially greater than -2.5.

The foregoing is offered primarily for purposes of illustration. It will be readily apparent to those skilled in the art that numerous variations, modifications and substitutions, in the materials and methods described herein may be made without departing from the scope of the invention.

IT IS CLAIMED:

1. A method for suppressing appetite in a mammal, which comprises:

administering to such mammal in need of appetite suppression an effective amount of a compound of formula (I) or a pharmaceutically effective salt thereof:



(I)

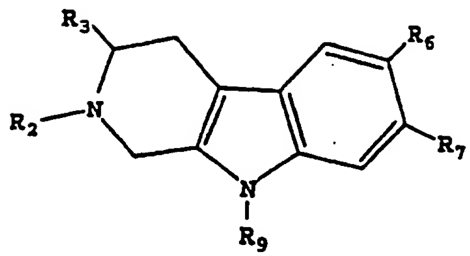
wherein R_2, R_3, R_6, R_7, and R_9 are so selected that the predicted activity, measured in terms of IC_{50}, as calculated by the formula $\ln(1/IC_{50}) = 0.058P^2 + 0.908(\sigma + \sigma_I) - 2.647$ is greater than about -2.5, where P is the octanol/water partition coefficient determined using the fragment method, σ is the sum of Hammet parameters for the R_6 and R_7 substituents, and σ_I is the sum of the Inductive parameters for the R_2 and R_3 substituents.

2. The method of claim 1, wherein $R_2 = R_7 = H$, R_9 is H, ethyl, benzyl, or n-pentyl, R_6 is methyl, Cl, Br, I, or H, R_3 is carboxyl or H, and the predicted activity as measured by $\ln(1/IC_{50})$ is greater than -2.1.

3. The method of claim 2, wherein R_9 is n-pentyl, and R_2, R_3, R_6, and R_7 = H.

4. A composition for suppressing appetite in a mammal, which comprises:

a pharmaceutically acceptable excipient, and an effective amount of compound (I):



(I)

wherein R_2, R_3, R_6, R_7, and R_9 are so selected that the predicted activity, measured in terms of IC_{50}, as calculated by the formula $\ln(1/IC_{50}) = 0.058P^2 + 0.908(\sigma + \sigma_I) - 2.647$ is greater than about -2.5, where P is the octanol/water partition coefficient determined using the fragment method, σ is the sum of the Hammet parameters for the R_6 and R_7 substituents, and σ_I is the sum of the Inductive parameters for the R_2 and R_3 substituents.

5. The composition of claim 4, wherein $R_2 = R_7 = H$, R_9 is hydrogen, ethyl, benzyl, or n-pentyl, R_6 is methyl, Cl, Br, I, CF_3, or H, and R_3 is carboxyl or H, and the predicted activity as measured by $\ln(1/IC_{50})$ is greater than -2.1.

6. The composition of claim 5, wherein $R_2 = R_3 = R_7 = H$, R_9 is n-pentyl, and R_6 is Cl, Br, I, H, CF_3, or methyl.

7. The composition of claim 6, wherein $R_2 = R_3 = R_6 = R_7 = H$, and R_9 is n-pentyl.

8. The composition of claim 6, wherein $R_2 = R_7 = H$, R_9 is n-pentyl, and R_6 is methyl, and R_3 is H.

9. The composition of claim 4, wherein $R_2 = R_3 = R_6 = R_7 = H$, and R_9 is benzyl.

10. The composition of claim 4, wherein $R_2 = R_3 = R_6 = R_7 = H$, and R_9 is ethyl.

11. The composition of claim 4, wherein $R_2 = R_7 = R_9 = H$, R_6 is H, CF_3, Cl, Br, or I, and R_3 is COOH or H.

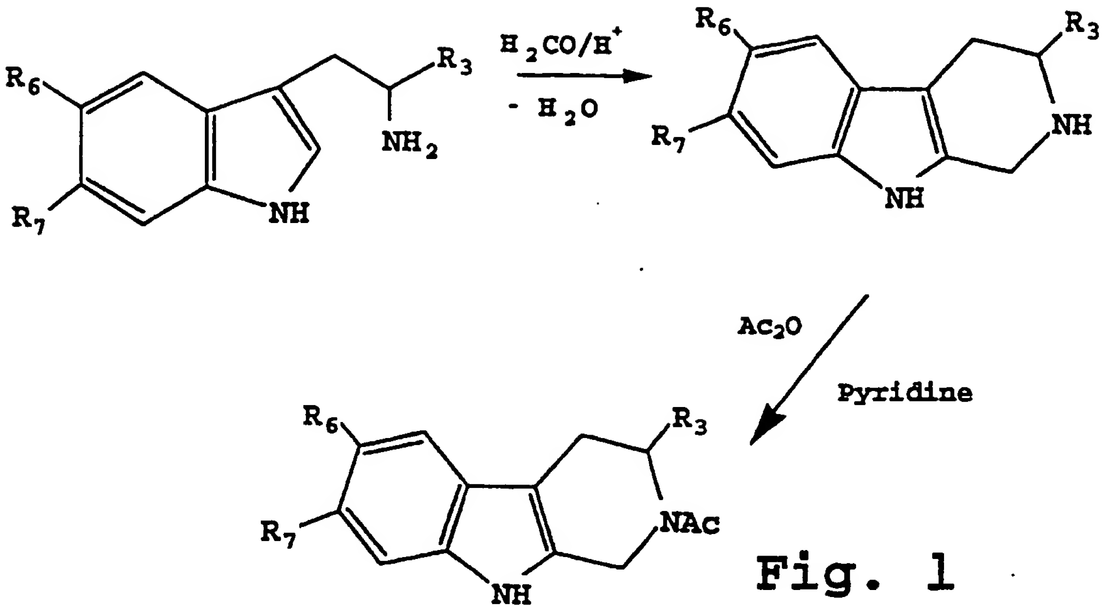

R6
R7
R3
NH2
NH
H2CO/H+
- H2O
Ac2O
Pyridine
NAc

Fig. 1

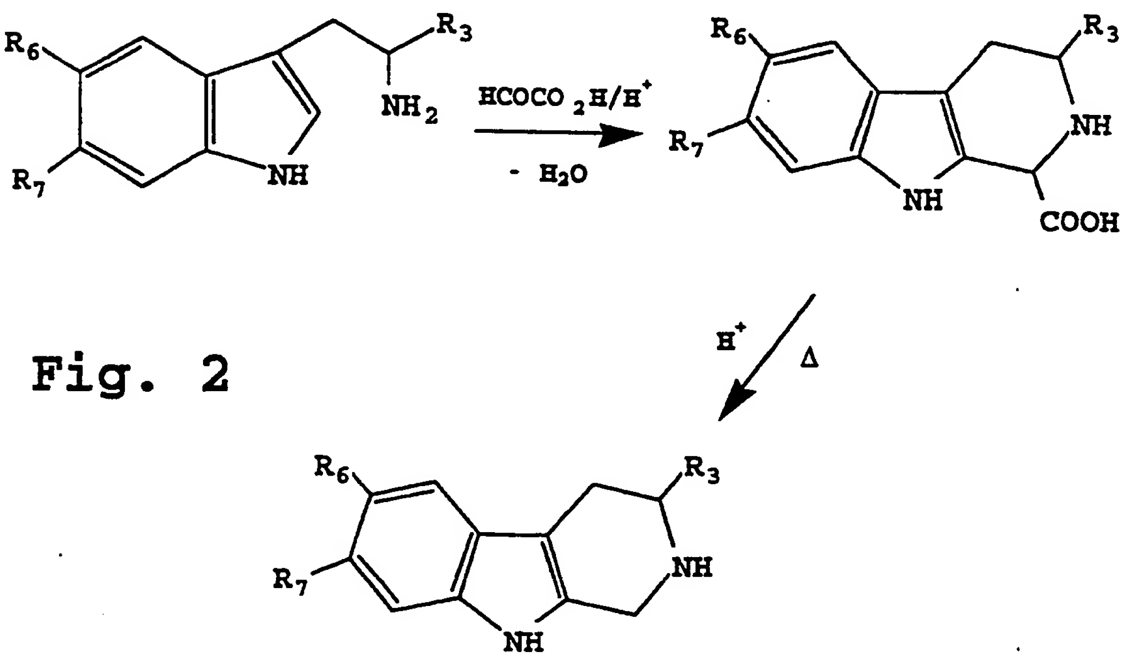

R6
R7
R3
NH2
NH
HCOCO2H/H+
- H2O
COOH
H+
Δ

Fig. 2

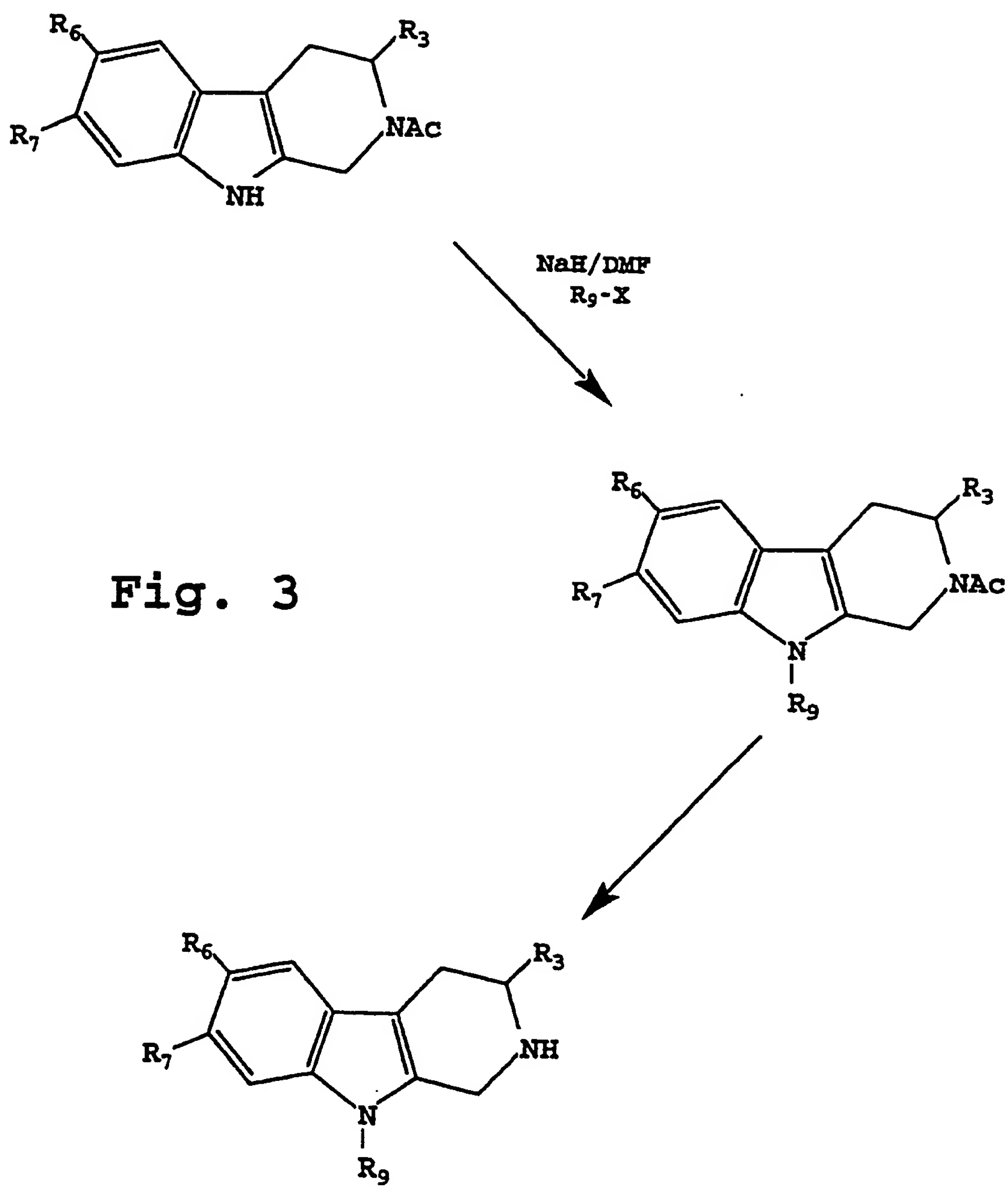


Fig. 3

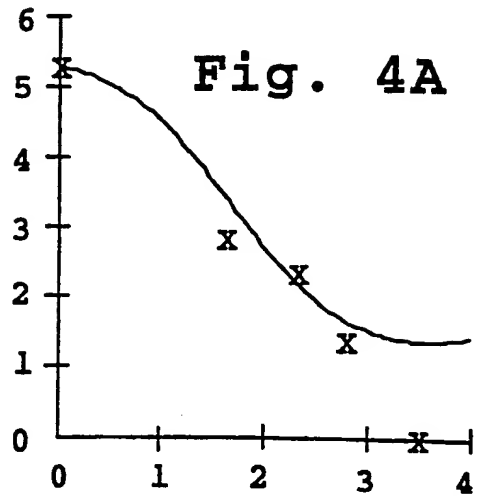


Fig. 4A
6
5
4
3
2
1
0
0
1
2
3
4

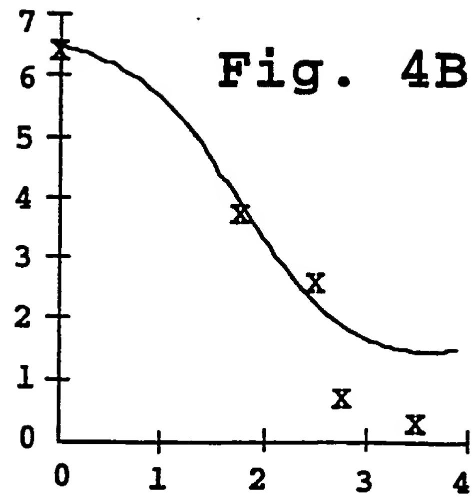


Fig. 4B
7
6
5
4
3
2
1
0
0
1
2
3
4

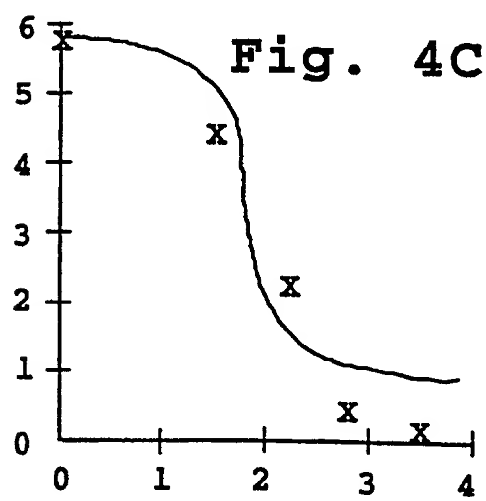


Fig. 4C
6
5
4
3
2
1
0
0
1
2
3
4

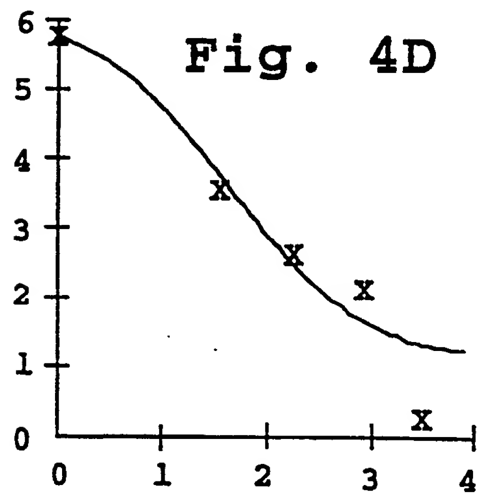


Fig. 4D
6
5
4
3
2
1
0
0
1
2
3
4

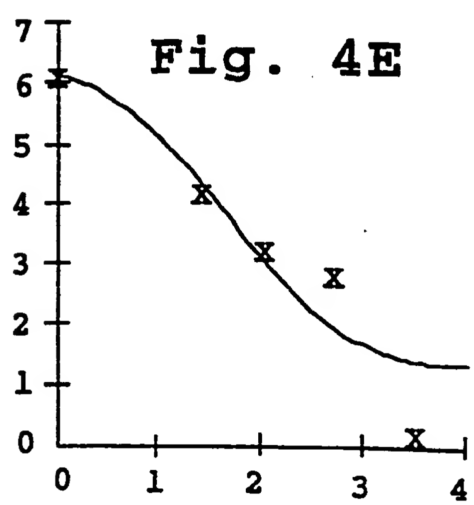


Fig. 4E
7
6
5
4
3
2
1
0
0
1
2
3
4

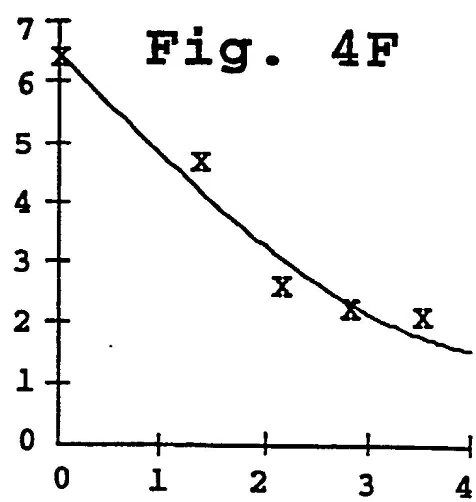


Fig. 4F
7
6
5
4
3
2
1
0
0
1
2
3
4

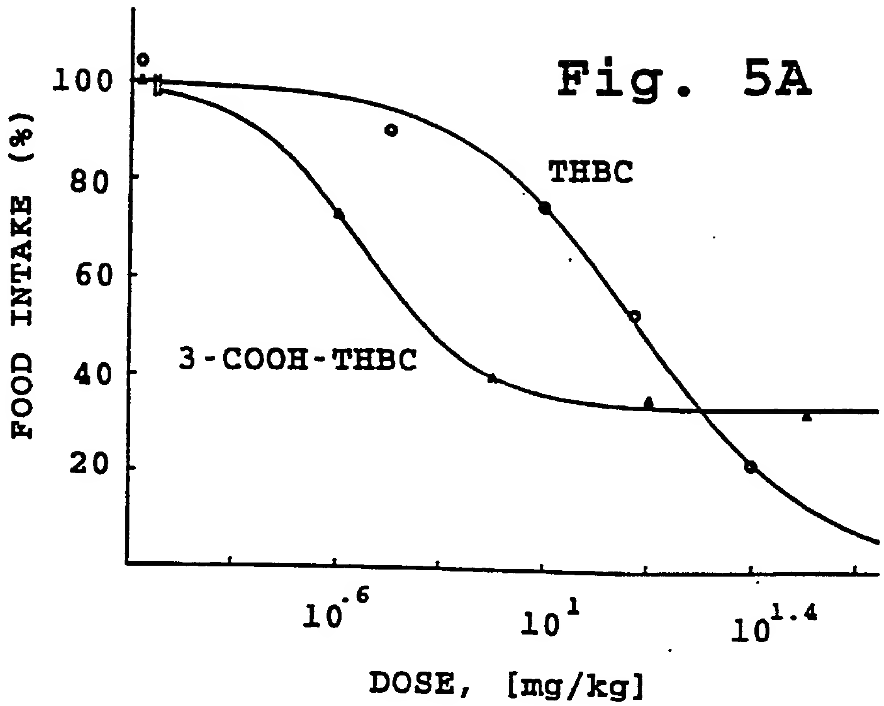

100
80
60
40
20
FOOD INTAKE (%)
THBC
3-COOH-THBC
$10^{.6}$
10^{1}
$10^{1.4}$
DOSE, [mg/kg]

Fig. 5A

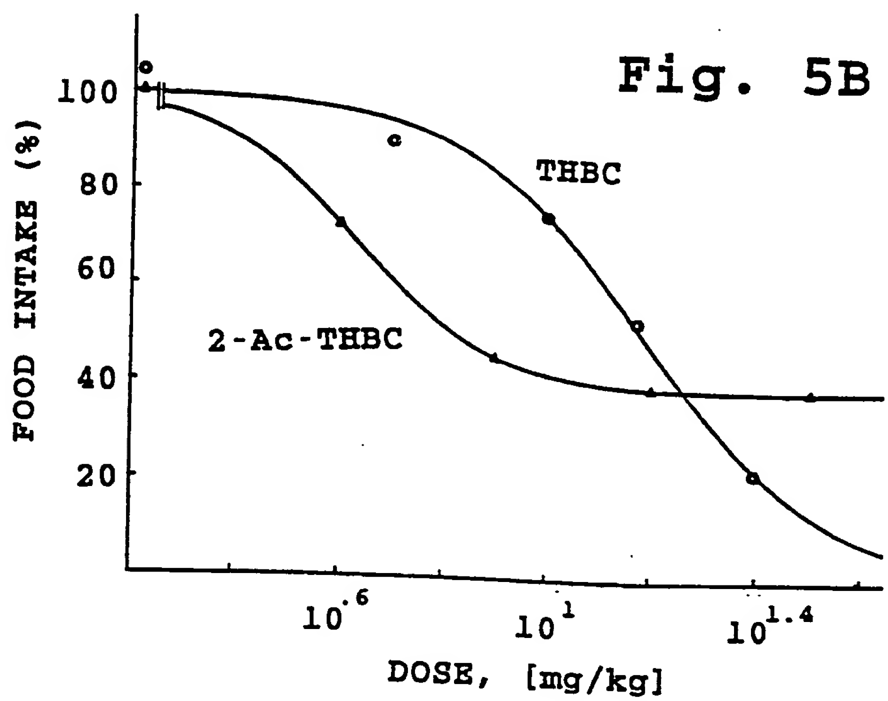

100
80
60
40
20
FOOD INTAKE (%)
THBC
2-Ac-THBC
$10^{.6}$
10^{1}
$10^{1.4}$
DOSE, [mg/kg]

Fig. 5B

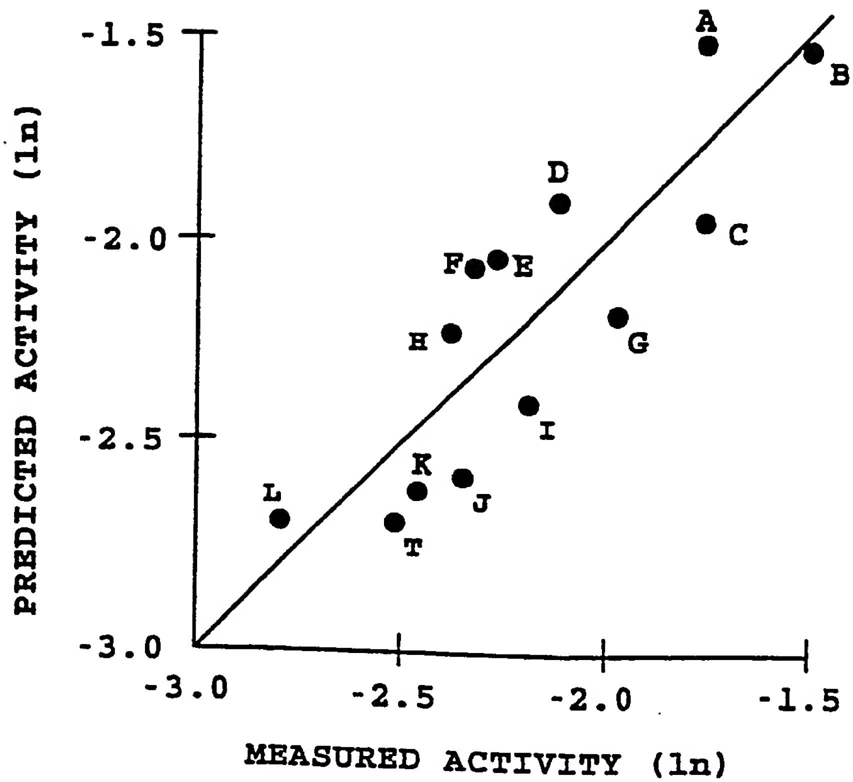
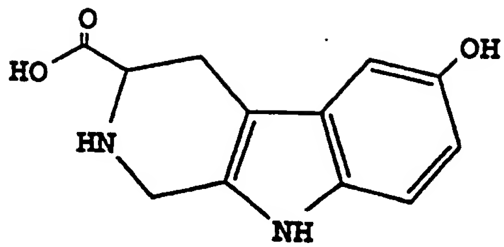

-1.5
-2.0
-2.5
-3.0
-3.0
-2.5
-2.0
-1.5
PREDICTED ACTIVITY (ln)
MEASURED ACTIVITY (ln)
A
B
C
D
E
F
G
H
I
J
K
L
T

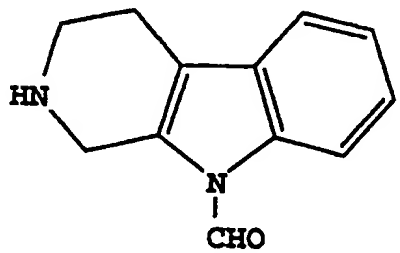
Fig. 6

Predicted ln(IC $_{50}$) (Observed)



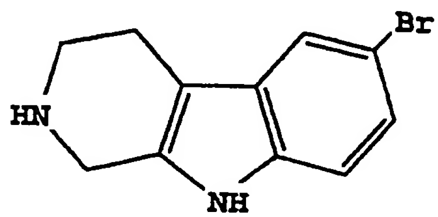
(M)

-2.62 (-3.07)



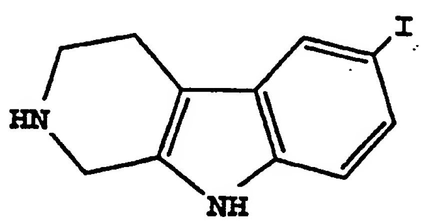
(N)

-2.65 (-2.98)



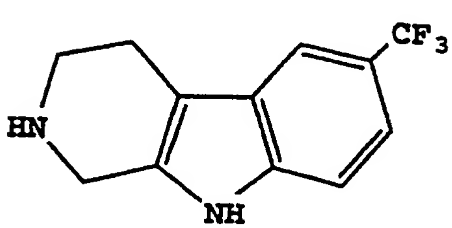
(O)

-2.06



(P)

-1.97



(Q)

-1.81

Fig. 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/03249

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5) A61K 31/44
US CL. 514/292,909 546/84

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	514/292,909 546/84

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X / Y	Cooper, et al. Pharmacol. Biochem Behav. 1986 25 (1) 99-106 Chemical Abstracts Völ. 105, 1986 Abstract: 127278 p	1 to 11 / 1 to 11
A	Glassman,et al Science 226. 1984 p. 883-6 (Entire document)	1 to 11
A	Dewar, et al JACS 84. 1962 pp 3548 to 3553 (Entire document)	1 to 11
A	EP,A 0,304,223, (EVANS) 22 February 1989 (Entire document)	1 to 11
A	Robertson, et al European J.Pharmacol 76 (1981) 281-284 (Entire document)	1 to 11

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
13 July 1990	20 AUG 1990
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Fn Edward C. Ward NGUYEN NGOC-HO INTERNATIONAL DIVISION Ngoc Ho Nguyen

International Application No. PCT/US90/03249

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X / Y	Rommelspacher, et al Naunyn-Schiedeberg's Arch Pharmacol. 298 83-91 (1977) p. 84 to 86	1-11 / 1-11

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ...... because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers__________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.